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CHEDDAR CHEESE FLAVOR. III. ACTIVE SULFHYDRYL GROUP PRODUCTION DURING RIPENING[1]

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SUMMARY

The influence of incubation and heat treatment of milk on the active –SH group concentration in Cheddar cheese was determined. Eight-month-old cheese manufactured from nonincubated and incubated (37 C for 5 hr) raw milk exhibited average –SH values (mg cysteine HCl equiv./100 g of cheese) of 3.5 and 13.0, respectively. Concentrations of active –SH groups in cheese manufactured from raw milk or from milk heated at 143 F for 5 and 30 min, and 155 F for 15 min, were related inversely to the severity of the heat treatment of the milk. Active –SH groups appeared in raw-milk cheese after one week of ripening, reaching maximum values after one to three months of curing. Heating the milk delayed the appearance of active –SH groups in the cheese during curing. Generally, the intensity of characteristic Cheddar flavor was related to the concentrations of active –SH groups of the cheese but not to bacterial numbers.

Preliminary results have indicated a possible relationship in Cheddar cheese of active –SH (sulfhydryl) groups and flavor (3). Therefore, the study was extended by an investigation of certain factors which affect the active –SH group concentration in Cheddar cheese as determined by the thiamine disulfide (TDS) method, with the view of attempting to establish more reliably the –SH group-flavor interdependance. The factors investigated were (a) incubation of the milk, (b) heat treatment of the milk, and (c) curing of the cheese.

EXPERIMENTAL PROCEDURES

Milk was obtained from two sources. For the study on the effect of incubation, uncooled mixed-breed milk was divided into two lots within 30 min following milking. Cheese was manufactured from one of these lots immediately, whereas the second lot was incubated at 37 C for 5 hr before cheese manufacture. This incubation treatment had been demonstrated previously to result in cheese of optimum flavor quality and intensity (4). Three series of cheese were manufactured.

For the study of the effect of heat treatment of the milk and curing time of the cheese, mixed-herd Grade A raw milk from a commercial supply was divided into four lots: (a) no treatment, (b) heated to 143 F for 5 min, (c) heated to 143 F for 30 min, and (d) heated to 155 F for 15 min. Three series of cheese were manufactured.

The cheese was manufactured according to the standard 4.5-hr procedure, paraffined, cured at 50 F for one month and then at 40 F. Quality evaluations for flavor, body and texture, and characteristic flavor (7) were made by three experienced judges.

Whey protein nitrogen determination of the milk was made according to Kuramoto et al. (9). Cheese analyses included bacterial estimates (6), thiamine disulfide reducing capacity (3), pH (7), and moisture by the infrared drying method.

RESULTS

Milk incubation. The effect of raw milk incubation on the formation of active –SH groups and flavor of the cheese is shown in Table 1. The formation of active –SH groups varied for the individual trials, but differences between the lots of cheese manufactured from nonincubated and incubated milk are clearly evident, particularly after eight months of curing. Two-month values were essentially the same for both types of cheese, approximating 5 mg cysteine HCl equiv./100 g. At eight months, the concentrations of active –SH groups remained low in the cheese manufactured from nonincubated milk, but increased to an average

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TABLE 1

Effect of incubation of fresh raw milk at 37 C on the concentration of active sulfhydryl groups and the flavor quality of Cheddar cheese [a]

Incubation time (*hr*)	Active -SH groups [b]				Flavor score and criticisms [c]				C.F. [d]			
	Two months		Eight months		Two months		Eight months		Two months		Eight months	
	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg
0	3.1-6.6	4.7	1.0- 7.7	3.5	36.5-40.0 a, m	37.8	36.0-37.5 a, b, m, l	36.7	0-2	1	0-2	0.7
5	2.5-7.2	5.5	12.7-13.2	13.0	38.0-40.0 a, b, u	38.8	38.5-39.0 b, s	38.7	1-4	2.3	5-6	5.3

[a] Three series.
[b] Expressed as mg cysteine HCl equivalent/100 g of cheese.
[c] a-acid; b-bitter; l-lacking; m-metallic; s-sulfide; u-unclean.
[d] C.F.—Characteristic Flavor: 0, missing; 1-2, very slight; 3-4, slight; 5-6, definite; 7-8, pronounced.

value of 13.0 in that manufactured from incubated milk.

The flavor of the cheese, expressed either as numerical scores or characteristic flavor intensity, was directly related to the concentrations of active -SH groups. After eight months, the flavor scores were about two points higher and the intensity of characteristic flavor five- to sixfold greater in the cheese manufactured from incubated milk compared to that from nonincubated milk.

The pH of the cheese ranged from 5.1 to 5.4, the moisture content from 34.6 to 37.0%, and body and texture scores from 28.0 to 30.0. These normal values were unaffected by the differences in milk treatment.

Heat treatment of the milk. In the heat-treatment phase of the study, attention was given to the effect of heating the milk on (a) the whey protein content of the milk, and (b) the formation of active -SH groups during cheese manufacture and ripening.

The whey protein content of the three different lots of milk was 0.89%. Of the three heat treatments used (143 F—5 and 30 min, and 155 F—15 min), only the latter affected the whey protein nitrogen values, reducing them by 15.7 to 35.7% below the initial results.

TDS-reducing determinations were made of the milk, whey, and curd at each step of the manufacturing process to the point of removing the pressed curd (approximately 15 hr following hooping). Active -SH groups were not detected during this period.

Results in Figure 1 reveal direct relationships between the heat treatment of milk and a) the concentrations of active -SH groups in the cheese, b) the slowness with which -SH groups appeared in the cheese during the early stages of curing, and c) the constancy of the -SH groups during curing.

The cheese manufactured from 155 F—15 min treated milk never developer active -SH groups. For the other cheeses the maximum average values were 11.7 for raw milk (two months), 9.5 for 143 F—5 min treated milk (two months), and 3.4 for 143 F—30 min treated milk (six months).

Active -SH groups first appeared in the raw milk cheese and in the 143 F—5 min treated milk cheese in about 1 wk. Concentrations of -SH groups varied widely in these lots of cheese during ripening, whereas the magnitude of variation was less in the 143 F—30 min treated milk cheese. The major difference between individual series of cheese was in respect to the rate of formation of the -SH groups. Maximum -SH group concentrations occurred

TABLE 2

Quality characteristics of Cheddar cheese manufactured from raw and heat-treated milk [a]

Milk treatment	Flavor score				Characteristic flavor [b]				Body and texture score			
	Three months		Six months		Three months		Six months		Three months		Six months	
	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg	Range	Avg
Raw	37.0-38.0	37.7	35.0-39.0	37.3	4.0-4.0	4.0	2.0-6.0	4.0	29.0-29.0	29.0	28.0-30.0	29.2
143 F— 5 min	37.5-37.5	37.5	37.5-38.0	37.7	2.0-2.0	2.0	3.0-4.0	3.5	29.5-29.5	29.5	30.0-30.0	30.0
143 F—30 min	36.0-37.0	36.5	36.0-37.0	36.7	1.0-2.0	1.3	0 -2.0	1.3	28.0-29.0	28.7	27.5-29.0	28.3
155 F—15 min	35.5-37.5	36.5	36.0-37.5	37.0	0	0	0 -2.0	0.7	28.5-28.5	28.5	28.5-29.0	28.7

[a] Average of three series.
[b] 0, missing; 1-2, very slight; 3-4, slight; 5-6, definite; 7-8, pronounced.

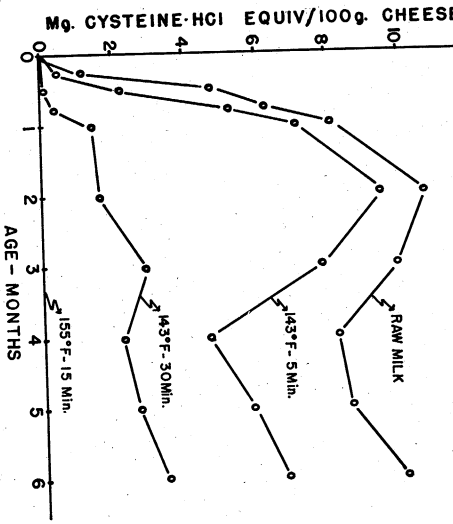

Fig. 1. Concentration of active sulfhydryl groups of Cheddar cheese manufactured from raw and heat-treated milk (average of three series).

in the raw milk lots between one and three months of curing and measurable –SH concentrations were present in the 143 F—30 min treated milk lots between 2 and 4 wk. The rate of –SH group formation was characteristic for the individual series, with all lots of cheese in a series behaving similarly in comparison of the cheese of other series.

Quality evaluations for the cheese from the raw and heated milks (Table 2) reveal an inverse relationship between the heat treatment of the milk and the flavor quality of the cheese. This is revealed more clearly by the characteristic flavor (C.F.) results than by the numerical scores. At six months, the cheese from the raw milk and mildly heated milk (143 F—5 min) had average C.F. intensities threefold greater than that from regular pasteurized milk (143 F—30 min) and five- to sixfold greater than that from milk heated at 155 F—15 min.

Overall, the body and texture scores ranged from 27.5 to 30.0 (Table 2). Generally, the cheese manufactured from raw milk and milk heated at 143 F—5min was superior in body and texture to that manufactured from milk receiving more intense heat treatment.

Average moisture contents of the cheese were 38.2% (raw milk), 38.1% (143 F—5 min), 39.2% (143 F—30 min), and 39.7% (155 F—15 min), which indicates a slight increase in moisture content with increasing heat treatment of the milk. The pH values (5.0-5.2) were normal for all of the cheese.

The bacterial counts of one-, three-, and six-month cheese (Table 3) reveal that cheese manufactured from raw milk contained considerably

Bacterial numbers and active sulfhydryl group concentrations in Cheddar cheese manufactured from raw and heat-treated milk [a]

Milk treatment	Bacterial counts (M/g) [b]			Active –SH groups [c]		
	One month	Three months	Six months	One month	Three months	Six months
Raw	22.0	11.0	13.0	9.8	9.0	8.6
143 F— 5 min	5.7	0.8	1.3	7.5	7.9	6.9
143 F—30 min	3.1	1.4	0.2	1.5	2.5	2.7
155 F—15 min	3.1	1.9	0.4	0	0	0

[a] Averages of two series. Bacteria were not enumerated in the third series.
[b] Millions/g.
[c] Expressed as mg cysteine HCl equivalent/100 g cheese.

higher numbers of bacteria than did the lots manufactured from heated milk. At six months, the cheese manufactured from raw milk averaged 13.0 million bacteria/gram, whereas the average bacterial counts of that manufactured from heated milk ranged from 0.2 to 1.3 million/gram. Overall, bacterial counts did not relate to the concentrations of active –SH groups.

DISCUSSION

The present study has revealed that active –SH groups are formed in Cheddar cheese during curing and that a definite relationship exists between the concentrations of –SH groups in the cheese and (a) the history and heat treatment of the milk supply and (b) the flavor quality of the cheese. That fresh raw milk and heat-treated milk generally result in cheese of relatively low flavor intensity is not new knowledge. However, it has not been demonstrated previously that the basic reason for the low flavor intensity may relate to correspondingly low concentrations of –SH groups, a relationship which may involve the reported effect of –SH groups on the cheese proteinase activity (10).

The mechanisms by which active sulfhydryl groups are formed, and the source of these compounds in Cheddar cheese, is a matter of conjecture only at this time. At the pH of Cheddar cheese, the Eo of the cystine-cysteine oxidation-reduction system would approximate −0.16 V. Thus, if redox potential were the only contributing factor, the sulfhydryl groups of cheese manufactured from raw milk should be completely activated at the completion of the manufacturing process when the redox potential of the cheese is −0.2 V or less (1, 5). The fact that active sulfhydryl groups do not appear during the manufacturing process would indicate that the cystine-cysteine system is bypassed in the establishment of the initial redox potential of the cheese. Furthermore, the time lag which occurs before active sulfhydryl groups do become apparent points out that the factors which contribute to the reduction of disulfide linkages are either missing or inactive in fresh cheese. These factors may involve proteolysis but, undoubtedly, include transport enzyme systems, since reduction of disulfide linkages is an energy-requiring process and spontaneous reduction is unlikely to occur. Regardless of their nature, the factors involved would appear to be extremely heat-labile, as indicated by the decrease in TDS-reducing capacities of the cheese manufactured from milk subjected to only slight heat treatment compared to that manufactured from raw milk. Certainly, when pasteurization of milk for cheese is practiced, the heat treatment should be kept at a minimum, to prevent extensive damage to the milk system.

Concerning the source of active sulfhydryl groups, the estimated whey protein content of the Cheddar cheese was 0.35 g per 100 g of cheese, based on the whey protein content of the milk (0.89%) and a moisture (whey) content of the cheese of 38%. The weighted cysteine potential of 0.35 g of whey protein would approximate 25 mg (2), which is the equivalent of a TDS-reducing capacity of 32. Thus, whey protein-contained cystine and cysteine could account for the TDS-reducing capacity of the cheese of the present study and, also, for the relatively high TDS value of 30 observed on samples of commercial cheese (3).

Of the heat treatments used for the milk, only the highest temperature (155 F—15 min) affected the whey protein content, and the effect was mild. Theoretically, then, if whey protein were the only consideration, the lots of cheese manufactured from the heated milk could have developed reducing capacities similar to those lots manufactured from raw milk.

It may be envisioned that the differences in active –SH group concentrations of the various lots of cheese arise from variations in bacterial

numbers and types. However, the results appeared to exclude bacterial numbers and possibly types from this consideration. In the case of the cheese manufactured from milk treated at 143 F for 30 min and 155 F for 15 min, the starter organisms would constitute the major portion of the bacterial flora.

The direct influence of the incubation of raw milk on the concentrations of active –SH groups in the Cheddar cheese may relate to the changing state of sulfhydryl groups in the milk as revealed previously (8). Cheddar cheese of optimum flavor quality results when fresh raw milk is incubated for 3.5 to 5 hr at 37 C (4). This period corresponds to the period of decelerated release of heat-produced sulfhydryl groups in milk (8). During this period, the sulfur groups of the whey protein in milk are postulated to change from the sulfhydryl to the disulfide state, a condition apparently necessary in milk if the sulfur groups are to function normally during the cheese-ripening process.

The incubation approach used in this and in a previous study (4), by which milk is held at 37 C for a period of time prior to its use in cheese manufacture, may offer a means by which many of the current difficulties in making quality cheese from the modern milk supply may be overcome. Reportedly, the present milk supply for use in Cheddar cheese manufacture, much of which is of Grade A quality, causes spasmodic problems in cheesemaking and rather consistently yields cheese which is lacking in full, characteristic flavor. The prompt and low-level cooling of the milk at the farm may prevent or retard subsequent changes in the sulfhydryl groups of the whey protein of the milk (8), which may be one aspect of this problem.

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